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Importance of vanadium and nutrient ionic ratios on the development of hydroponically grown cuphea

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Abstract

Cuphea (*Cuphea viscosissima* × *C. lanceolata* 'PSR 23') seed contains oils that have industrial application. However, little is known regarding cuphea's optimal mineral nutritional requirements or responses to inhibiting elements. Oil seed crops often need additional phosphorus (P) to achieve optimal economic yield. Vanadium (V), a commonly occurring soil constituent, interferes with plant P uptake and earlier work showed that V is a factor in lipid metabolism. Hydroponic culture was used to evaluate the relative effect of V on the development of cuphea. Relative root length, root surface area, root weight, and aerial dry weights decreased exponentially as the V concentration increased from 0 to 153 μM. In contrast to field observations of other crops, additions of $MgSO_4$ to increase the Mg:(Mg + Ca) ratio further decreased plant growth by as much 50% at V concentrations greater than 31 μM. Root length was decreased by about 50% of the control when the plant was grown in 153 μM V and relative root area and dry weight were decreased by ≥75%. Increases in V concentration sharply reduced secondary and higher order lateral branching. Reduction in root growth was accompanied by a general chlorotic appearance. The results suggest that readily available V in field situations will result in poor root growth and crop performance. Also, the interaction of V and $MgSO_4$, common in soils in the region, will lead to further reductions in yields in the field.
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Keywords: Magnesium; Roots; Shoots; Root area; Chlorophyll

1. Introduction

Cuphea (family Lythraceae) is a potential commercial crop because of the high C-8 to C-14 fatty acid content in its seed (Kleiman, 1987). *Cuphea viscossissima* grows in the eastern US and *C. lanceolata* in central Mexico, both have potential for domestication as oilseed crops (Graham and Knapp, 1989). Cuphea is an indeterminate flowering plant that can be cultivated to maturity in the northwestern Corn Belt (Gesch et al., 2002). However, little is known about its nutrient requirements. Most of the lipid is contained in the seed embryo, and a single fatty acid may represent as much as 94% of the total lipid content (Graham and Kleiman, 1992). Thus, factors that affect seed embryo numbers and size at maturity are of interest.

One nutritional factor, the resin-extractable V: (V + P) molar ratio in soil, is of particular interest because P is often a limiting nutrient in oil seed production and V may compete with P for accumulation (Bowman,

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1983; Olness et al., 2000). Interference effects of V have been observed for a number of compounds including lipids (Khan and Malhotra, 1987; Byczkowski and Kulkarni, 1992).

After its discovery in the early 1800s (Hammond, 1999), Witz and Osmond (1886) showed that V is very toxic to plants. Aside from noting its toxicity to plants, little work was done on V before the 1950s (Bertrand, 1950). Studies suggested that V enhanced growth at concentrations of about 10 ng V g^{-1} soil (Bertrand, 1950). Interest intensified when Arnon and Wessel (1953) concluded that V was essential for some plants. Subsequent studies showed that V is generally toxic to terrestrial plants at greater than pico-molar levels (Warington, 1954; Singh, 1971; Hara et al., 1976; Wallace et al., 1977; Davis et al., 1978).

In an extensive survey of surface soils in the US, Shacklette et al. (1971) found that about 14% of all sites contained more than 120 μg g^{-1} of V and at least 60% of all sites contained more than 38 μg g^{-1} of V. Recently, we observed that resin extractable V inhibited a maize hybrid and several varieties of soybean and wheat grown under field conditions (Olness et al., 1998, 2000, 2001, 2002; Olness, 2001). Because of the widespread distribution of V, we can reasonably expect that V may affect plant growth on cropland in the US. Because the content of V in soil has received little attention, we cannot identify soils or locations that might experience V toxicity.

Interest in V shifted from plant studies to molecular biology when Cantley et al. (1977) and Quist and Hokin (1978) showed that V was a potent inhibitor of Na^{+}/K^{+}-ATPase. Bowman (1983) showed that V was competitive with phosphorus for accumulation by *Neurospora crassa* L. This latter observation is consistent with earlier results (Bowman, 1983) and suggests that the effect may be more widespread than previously realized. Growth data from Singh (1971), Hara et al. (1976), Wallace et al. (1977), and Davis et al. (1978), which showed a marked inhibition of growth, provided a similar fit to the General Energy Model for Limited Systems when evaluated as a V:(V + P) molar ratio (Olness et al., 2000).

An apparent mitigation of the resin-extractable V:(V + P) effect by the Mg:(Mg + Ca) molar ratio has been noted in one maize hybrid and one soybean variety (Olness et al., 2001, 2002). The effect (about a 20% increase in seed yield), while important, seems indirect. Field-grown cuphea in west central Minnesota develops a poor root system and shows poor water use efficiency (Sharratt and Gesch, 2002). While poor root growth could be attributed to several elements (i.e., Cd, Cr, or Ba), V is also present in the area. The potential for enhanced Mg nutrition and concern for V sensitivity led us to study these factors in a growth chamber experiment. Our objectives were to determine the relative sensitivity of a cuphea hybrid (‘PSR-23’ *Cuphea viscosissima* × *C. lanceolata*) to V and Mg using hydroponic culture.

2. Methods and materials

Individual cuphea plants were grown hydroponically in a Conviron[1] growth chamber (Controlled Environ Ltd., Winnipeg, Manitoba, Canada) for a period of 28 days. Chambers were maintained at 28 °C day (14 h) and 20 °C night (10 h) temperatures. Temperature was monitored on an hourly basis. Cuphea seeds (5–10) were germinated with water on expanded vermiculite (1–2 mm particle diameter) contained in cup-shaped #4 polyethylene stoppers. The base of the stopper had been removed and covered with polyethylene mesh (2-mm opening) to permit passage of roots. When roots had reached about 10 mm, plants were thinned to one per cup, and the cup was inserted into the mouth of 125-ml polyethylene bottle containing nutrient solution. The exterior surfaces of the bottles were painted black to prevent light entry. Each bottle was fitted with a 3-mm grommet and 2-mm internal diameter polyethylene aeration tube to maintain an oxidized solution. Air was continuously bubbled through the solution using a manifold system. Bottles were distributed randomly within the growth chamber. Nutrient solutions were exchanged on 48-h intervals.

The experiment was conducted as two trials and each had four replicates of each treatment. The final result thus provided eight replicates of each treatment. Two $MgSO_4$ (0.346 and 1.38 mM) and seven vanadate (0.00, 15, 26, 41, 61, 102, and 153 μM) concentrations

[1] Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

were used. The remaining concentrations of nutrients in the culture solutions are given in Table 1. Culture solution pH was 6.75 ± 0.05. The experimental design was a randomized complete block with [V] treatments and [Mg] subtreatments.

Shoot and root lengths were measured manually daily. Five chlorophyll measurements were made on the uppermost fully expanded leaves at 28 days using a Minolta SPAD-502 (Konica Minolta Business Solutions USA Inc., Ramsey, New Jersey). Root and shoot areas and surface areas were measured using a Li-Cor model 3100 area meter (LI-COR Inc., Lincoln, Nebraska). Roots and shoots were separated at harvest, dried at 60 °C, weighed, and then ground. To

Table 1
Nutrient solution for cuphea

Chemical	Concentration (mM)	Chemical	Concentration (μM)
Ca^{2+}	1.24	VO_3^-	0, 15, 26, 41, 61, 102, 153
$MgSO_4$	0.346, 1.38	Mn	5.22
K^+	7.19	Zn	0.441
NH_4^+	3.17	Cu	0.199
SO_4^{2-}	0.705, 2.085	Fe	10
NO_3^-	5.56	EDTA	10.4
HPO_4^{2-}	3.23	Cl	10.4
		B	26.4
		Mo	0.287

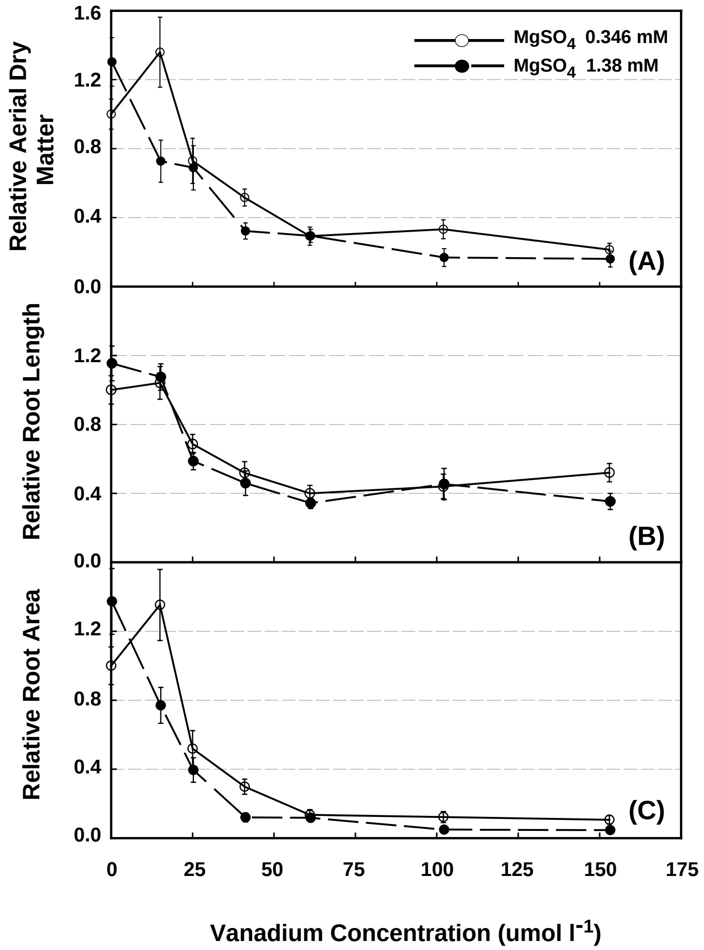

Fig. 1. Relative (A) aerial dry weight, (B) root length, and (C) root area of cuphea grown hydroponically for 28 days as a function of V. Molar Mg:(Mg + Ca) ratios were 0.22 or 0.89. Vertical bars represent standard errors of the means.

aid comparison between plants grown during separate trials, the mean values of the control treatments (without V and with 0.346 mM Mg) were set equal to 1 and all other production was measured as a fraction of this amount. SAS PROC GLM was used to evaluate the data (SAS Institute, 1989).

3. Results and discussion

A very marked V by $MgSO_4$ interaction is apparent in the data for the relative aerial dry weights (Fig. 1A, $P < 0.05$). At a Mg:[Mg + Ca] molar ratio of 0.22, a 15 μM concentration of V appears to enhance aerial dry weight, but growth is clearly depressed at an exponential rate as the V concentrations increased beyond about 15 μM. Increasing the $MgSO_4$ concentration to 1.38 mM increased aerial dry weights in the absence of V, but either depressed or had no effect in the presence of even the smallest amounts of V. At the largest concentrations of V and $MgSO_4$, total aerial dry weights were only about 20–25% of those of the respective control treatments without V. The V:[V + P] molar ratios achieved in the experiment were ≤0.04. Similar V:[V + P] molar ratios were obtained in resin extractions of soils (Olness et al., 2000). However, the effective soil concentrations may be affected by the variety and concentrations of extractable elements.

Similar patterns between the aerial dry weights and relative root area were present (Fig. 1C; $P < 0.05$). Addition of $MgSO_4$ in the absence of V increased total root area by nearly 40%. However, with even the smallest amount of V, the root area was depressed by >20% relative to the control treatment and by nearly 50% compared with the treatment without V. While V inhibits cell division (Meisch and Benzschawel, 1978) and root growth, the interaction with $MgSO_4$ (probably an interaction with Mg) is perplexing. Within the cell, the redox potential is sufficient to produce the VO^{2+} ionic form and some competition with cations might be expected. However, competition between Mg^{2+} and V would be expected to diminish the negative effect of V on root growth and development when the Mg concentration is increased.

In view of the effect of V on root morphology, it is not surprising that relative root length (the distance from the stem to the end of the longest root) is affected strongly by V (Fig. 1B). However, relative root area is affected more severely than relative root length. Even at the greatest concentrations of V, relative root length remains about 30–45% of the controls, whereas root area was less than 20% of the controls.

The effect of V on root dry weight was less than its effects on relative root length and relative root area

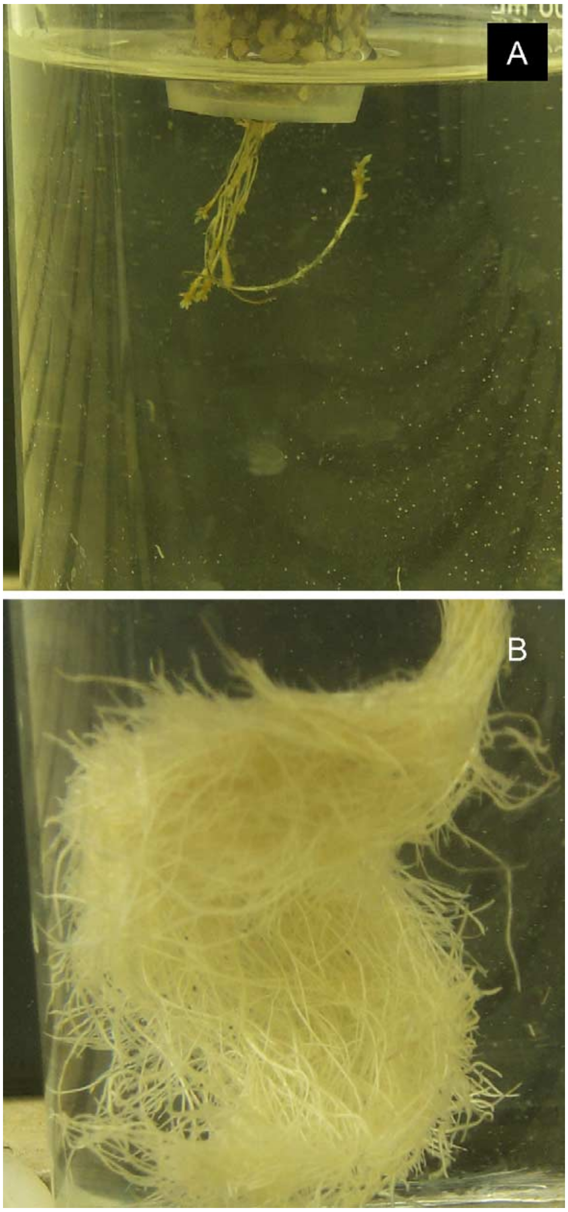

Fig. 2. Cuphea roots grown hydroponically for 28 days (A) with vanadium and (B) without vanadium.

(data not shown). As the secondary and tertiary lateral branching decreases, thickness of the primary root increases (Fig. 2). Thus, in the presence of V, total root mass increased at a much slower rate as the plant grows.

The apparent sensitivity of cuphea to V is much greater than that found by Singh (1971) for maize, Hara et al. (1976) for cabbage, Wallace et al. (1977) for beans, or Davis et al. (1978) for barley, but very similar to that obtained by Warington (1954, 1956) for pea, soybean, and flax. The reason for the differences in sensitivities to V in these various studies is unknown. At less than millimolar concentrations, most vanadate exists mainly in the monomeric form. Under neutral conditions, vanadate dimers, tetramers and pentamers form and the extent to which the anion forms oligomers is affected by the pH, nutrient composition, and ionic strength of the solution (Pettersson and Elvingson, 1998). Plants may be more sensitive to one or more of the oligomeric forms, and differences in their relative intensity may explain the differences obtained by the various research groups. In vitro, the vanadate dimer inhibits phosphoglycerate mutase (Crans, 1994) and the tetramer inhibits enzymes in the pentose phosphate shunt (Crans and Schelble, 1990).

The most dramatic and visible effect of V on the growth of cuphea is the inhibition of secondary and tertiary root formation. One of the effects of V is an inhibition of mitotic cell division (Meisch and Benzschawel, 1978), and this effect is expressed clearly in cuphea and other plants as a diminution in the formation and development of root tips. At elevated concentrations of V, the effect results in a nearly branchless root (Fig. 2A). In the absence of V, the plant develops a fine fibrous root system (Fig. 2B). In addition to the well-documented inhibition of ATPase and P transfer enzymes, V interferes with enzymes involved with cell division to the point that multinucleate cells develop (Meisch and Benzschawel, 1978).

Inhibition of root development, which is coupled with poor translocation of V from the root to the shoot (Hara et al., 1976), is manifested as typical Fe-chlorosis. The youngest leaves developed a chlorotic appearance typical of inadequate Fe or S supply. Chlorosis, as an indication of overall development, was closely correlated with a near exponential decrease in chlorophyll (Fig. 3) as the V concentration increased, but the relative effect of V measured with the SPAD[1] meter was less intense than that on root or shoot growth. Thus, under field conditions, an

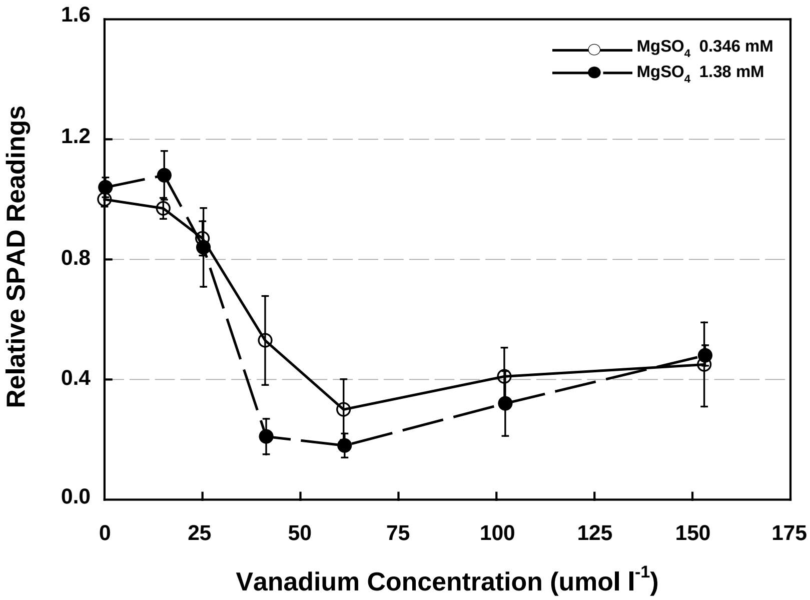

Fig. 3. Relative chlorophyll meter readings of leaves of cuphea grown hydroponically for 28 days as a function of V. Molar Mg:(Mg + Ca) ratios were 0.22 or 0.89. Vertical bars represent standard errors of the means.

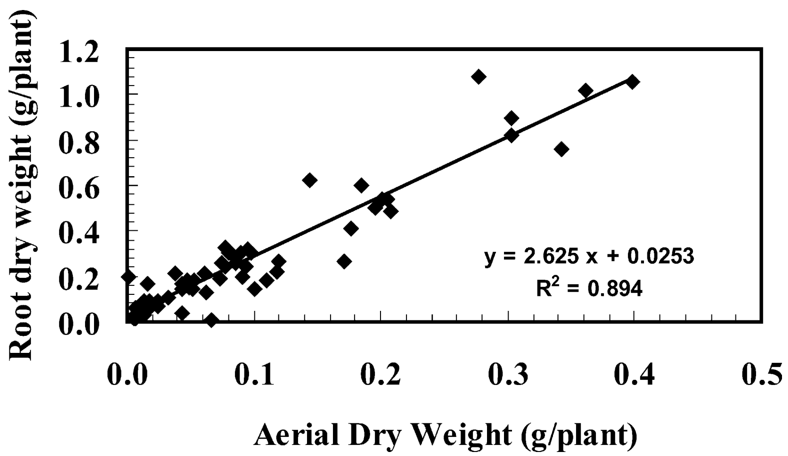

Fig. 4. The relationship between aerial and root growth of Cuphea grown hydroponically for 28 days.

effect of excess V may often be expressed as a slower growth rate instead of a chlorotic plant, particularly in environments rich in accessible Fe and S. This latter effect is consistent with the close correlation between shoot and root growth (Fig. 4). An analysis of the residuals of a linear model show a slight departure at greater growth rates suggesting a curvilinear function describes the relationship.

Shacklette et al's. (1971) observations on surface soils serves as a warning that potential problems with V exist across the US. To date, no systematic examination has been conducted to identify those sites where refined management (rhizosphere, fertility, or genotype) effects more efficient use of the soil resource.

4. Conclusions

Hydroponically grown cuphea seems as sensitive as most crop plants to micro- or milli-molar concentrations of V in the nutrient solution. Growth rates of both shoot and root portions decrease exponentially as the V concentration increases. Increasing the Mg concentration in solution increased the sensitivity of cuphea to toxic levels of V. The visible symptoms of V toxicity were stunting of shoot growth accompanied by chlorosis of the upper most leaves and a loss of secondary and tertiary branching of the root system. During the early seedling growth-stage, shoot growth was correlated closely with root growth. Increasing the Mg:(Mg + Ca) molar ratio from 0.22 to 0.89 had no measurable effect on root morphology or relative growth rate.

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